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UREA UV

STABLE LIQUID

CE

Cat. No.:	616663	616664	616665
	120 ml (1x90 ml+1x30ml)	500 ml (1x375 ml +1x125 ml)	10x20 ml (10x15 ml+ 10x5 ml)

Reagent kit for determination of urea concentration in serum and urine. Enzymatic (UV) method.

The detoxification of $NH4^+$ formed in the catabolism of amino acids takes place in the urea cycle. Enzymes catalyzing these reactions are synthesized in the liver. The end product is Carbamide (Urea) which is a nontoxic, nonpolar, small molecule. It is eliminated by the kidney. Increased levels are associated with renal diseases, as well as dehydration, circulatory collapse gose, gastrointestinal hemorrhage and diabetic coma. Decreased values are observed in some cases of severe liver disease.

Principle

Ammonia and Carbon dioxide (CO_2) are produced when urea is hydrolyzed in presence of Urease. The Ammonia produced in the reaction combines with 2-Oxoglutarate and NADH in the presence of Glutamate dehydrogenase (GLDH) to yield glutamate and NAD^+. The NADH/NAD^+ reaction produces a unique change in absorbance at 340 nm, which correlates with the concentration of urea nitrogen in the sample.

$$Urea + H_2O + \xrightarrow{\text{UREASE}} 2NH_4^+ + CO_2$$

$$2NH_4^+ + 2\text{-}Oxoglutarate + 2NADH \xrightarrow{\text{GLDH}} 2L\text{-}Glutamate + 2NAD^+ + 2H_2O$$

Reference values

Serum: **2.49-7.47 mmol/l** (15-40 mg/dl)
Urine: **333-583 mmol/24 h** (20-35 g/24 h)
It is recommended that each laboratory should assign its own normal range.

Reagents

1. Reagent (R1)

NADH	320 µmol/l

2. Reagent (R2)

Tris buffer, pH=7.60	100 mmol/l
α-Ketoglutarate	9 mmol/l
Urease	6500 U/l
GLDH	1100 U/l

Avoid direct exposure to light.

Precaution

These reagents contain 0.1 % sodium azide. To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent.

Sample

Serum free of haemolysis.
Urine diluted in ratio of 1:100 with distilled water. Do not use anticoagulants containing fluoride or ammonium ions!

PROCEDURE

Preparation and stability of working reagent

• One-reagent procedure
Mix 3 volumes of reagent (R1) with 1 volume of reagent (R2). Stability:

at 20-25 °C:	7 days
at 2- 8 °C:	4 weeks

• Two-reagent procedure
Reagents are ready for use.
If the absorbance of working reagent is lower than 1.5 at 334 nm the reagent can not be used.

Assay conditions

Wavelength:	340 (334-365) nm
Temperature:	37 °C
Cuvette:	1 cm light path
Read against:	distilled water
Method:	kinetic (decreasing)

• **One- reagent procedure**

Working reagent	1,0 ml
Sample	10µl

Mix and after 30 seconds incubation measure the absorbance during 1 minute.

• **Two-reagent procedure**

R1	1,5 ml
Sample	20 µl

Mix and after 60 seconds incubation then add:

R2	500 µl

Mix and after 30 seconds incubation measure the absorbance during 1 minute.

Calibration (37°C, UV method)
S1: Distilled water
S2: Urea standard Cat. No.: 850811 or
Roche C.F.A.S. (Calibrator for automated system)
Randox Calibration Serum Level I or
Randox Calibration Serum Level II

Calibration frequency

Two-point calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} x C_{standard} = C_{sample}$$

A = Absorbance, C = Concentration

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 600 analyser (37°C).

Linearity

The test is linear up to 66.7 mmol/l (400 mg/dl).

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 0,009 mmol/l (0,05 mg/dl) Urea concentration at 334 nm.

Precision

	Reproducibility		
Sample	Average concentration (mmol/l)	SD	CV%
Sample I.	7.6	0.25	3.26
Sample II.	25.6	0.90	3.51

	Repeatability (twenty replicates)		
Sample	Average concentration (mmol/l)	SD	CV%
Sample I.	7.4	0.12	1.58
Sample II.	23.1	0.30	1.29

Correlation

Comparative studies were done to compare our reagent with our Urea UV powder reagent.
The results from these studies are detailed below.
Correlation coefficient: r =0.9994
Linear regression: y (mmol/l)= 0.972x+0.710
(x= powder reagent, y= liquid reagent).

Specificity

Bilirubin 855 µmol/l (50mg/dl), lipid 1000 mg/dl, glucose 55.5 mmol/l (1000mg/dl) and ascorbic acid 2.84 mmol/l (50mg/dl) don't interfere with the assay at the given levels.

Note

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

LOT Batch Code

REF Code

Bibliography

Talke H,. Schubert G.E. Klin.1965, 43:174.